REMARKS

Applicant requests reconsideration of the application in view of the foregoing amendments and the discussion that follows. The status of the claims is as follows. Claims 41-97 were previously canceled without prejudice to Applicant's filing of divisional applications to the separately patentable subject matter thereof. Claims 1-40 and 98-101 stand rejected. Claims 1, 25, 98 and 100 were amended herein.

Applicant wishes to thank Examiners Mahatan and Allen for the courtesy of a telephonic interview on February 11, 2004, with a follow-up with Examiner Mahatan on February 19, 2004, in which the above amendments to the claims were discussed. Examiner Mahatan indicated that he would prepare an interview summary and forward it to Applicant. No agreement was reached.

The Amendment

Claim 1 was amended to recite that the hybridization of the hybridization oligonucleotide is predicted by the presence of the hybridization oligonucleotide in the cluster. Support therefor is in the Specification, for example, page 29, lines 16-17, page 30, lines 25-27.

Claim 25 was amended to incorporate the subject matter of original Claim 93, now canceled.

Claims 98 and 100 were amended in a manner similar to that for Claim 1.

Terminal Disclaimer

Applicant acknowledges the indication in the Office Action that the Terminal Disclaimer filed October 3, 2003, has been approved.

Rejection under 35 U.S.C. §112

Applicant submits that the above amendment to Claim 25 obviates this ground of rejection.

Rejection under 35 U.S.C. §102

Claims 1-3, 5-10, 15, 17-22, 98 and 100 were rejected under paragraph (b) of the above code section as being anticipated by Hyndman, *et al.* (Hyndman).

Hyndman does not anticipate the methods of Claims 1-3, 5-10, 15, 17-22, 98

and 100. As Applicant indicated previously and as the Office Action has recognized, the present methods are based on Applicant's discovery that oligonucleotides showing hybridization tend to form clusters. Applicant's claimed methods reflect this discovery in that the claims recite the step of selecting, for a cluster, a hybridization oligonucleotide wherein the hybridization of the hybridization oligonucleotide is predicted by the presence of the oligonucleotide in the cluster. The Hyndman reference is completely devoid of any teaching in this regard and, as such, Hyndman does not disclose each and every element of the claimed invention. Accordingly, a *prima facie* case of anticipation has not been established. *In re Paulsen*, 30 F.3d 1475, 1478, 31 U.S.P.Q.2d 1671, 1673 (Fed. Cir. 1994).

Hyndman discloses software to determine optimal oligonucleotide sequences based on hybridization simulation data. The authors indicate that their new computer program, HYBsimulator™ (formerly OligoProbe DesignStation) creates a set of candidate oligonucleotides from a target gene. For each of the candidate oligonucleotides, a large sequence database is searched for sequences that will hybridize to the oligonucleotide. The authors refer to this as computer hybridization simulation (CHS). Using the nearest-neighbor model, the HYBsimulator takes into account mismatches in hybridization and calculates the melting temperature or free energy for hybridization to all sequences in a database. The specificity of each oligonucleotide is then quantified by the number of genes that may hybridize and the predicted melting temperatures or free energies of hybridization to those genes. The CHS data are used to select oligonucleotides based on their specificity with respect to a database. As Hyndman explains, HYBsimulator creates a ProbeSet where the set contains all possible oligonucleotides derived for the target sequence that fit a chosen specification (page 1091, column 2, lines 14-17). Designated oligonucleotides are selected from the ProbeSet for a particular application. The specificity of probes in the ProbeSet is determined based on CHS data. CHS simulates hybridization of each probe in the ProbeSet with every sequence in a specified GenBank database. HYBsimulator performs multiple calculations for the possible sub-sequences and then selects the most favorable value (paragraph bridging pages 1091 and 1092).

Referring, for example, to Claim 1 as a typical claim, Claim 1 recites that a predetermined number of non-identical oligonucleotides within a nucleotide

sequence that is hybridizable with the target nucleotide sequence is identified. The oligonucleotides are chosen to sample a length of the nucleotide sequence. For each of the oligonucleotides at least one parameter that is predictive of the ability of each of the oligonucleotides to hybridize to the target nucleotide sequence is determined and evaluated. A subset of oligonucleotides within the predetermined number of non-identical oligonucleotides is selected based on an examination of the parameter. Then, oligonucleotides in the subset are identified that are in clusters along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence. A hybridization oligonucleotide is selected from a cluster where the hybridization for a hybridization oligonucleotide is predicted by the presence of the oligonucleotide in the cluster. The present invention avoids the evaluation of every probe in a probe set as carried out by Hyndman.

The Office Action argues that Hyndman describes the application of the HYBsimulator™ to identify common/census probes and equates this description to the language of Claim 1, namely, "clustered along a region"). The Office Action argues that the common/census probes are identified to all the targets of interest, i.e., same gene in related organism or related genes in the same organism or unknown varieties of a gene family. (citing the paragraph entitled "Common or Census Probes").

In the section referred to by the Office Action, Hyndman is concerned with detecting the same gene in related organisms or related genes in the same organism or to detect unknown varieties of a gene family by using a probe that identifies all know types of a particular gene family. To this end Hyndman's goal is to design a probe that detects a maximum number of the aforementioned genes. The probe must be effective under a multitude of circumstances. As Hyndman explains, in addition to his basic design (discussed above as to its lack of relevance to the present invention), users perform an additional CHS against a small database comprised of all the target genes. Hyndman evaluates a number of genes with regard to one probe.

On the other hand, again referring to present Claim 1 as an example, oligonucleotides in a subset are identified that are in clusters along a region of a nucleotide sequence that is hybridizable to the target nucleotide sequence, for example, a gene. This approach is the opposite of what Hyndman might be argued

to teach. Hyndman's approach is in line with his stated goal of finding a single probe that is effective against a large number of genes from a number of sources. Even in this aspect of Hyndman's teaching, CHS is employed. As explained above, CHS is a method that simulates hybridization of each molecule of interest, in this case one probe against as many of a multitude of genes as possible. The present invention, on the other hand, seeks to select good probes without performing full thermodynamic and other studies. Applicant has found that good probes can be obtained by viewing clustering of a multitude of probes along a region of a nucleotide sequence that is hybridizable to the target nucleotide sequence where the hybridization of a hybridization probe is predicted by the presence of a hybridization oligonucleotide in the cluster.

Claims 1-10, 15-17, 21, 23, 28, 37, 39, 40, 98 and 100 were rejected under paragraph (b) of the above code section as being anticipated by Rychlik, *et al.* (Rychlik). The reference discloses a method for choosing optimal oligodeoxyribonucleotides as probes for filter hybridization, primers for sequencing, or primers for DNA amplification. Three main factors that determine the quality of a probe are considered: stability of the duplex formed between the probe and target nucleic acid, specificity of the probe for the intended target sequence, and self-complementarity. DNA duplex stability calculations are based on the nearest-neighbor thermodynamic values determined by Breslauer, *et al.* Rychlik indicated that calculations for specificity of the probe and its self-complementarity were based on a simple dynamic algorithm. On page 8543, last paragraph, Rychlik states that a critical component of any such computerized method is the algorithm for determination of the duplex dissociation temperature T_d. On page 8544 the author indicates that the most precise methods for computing helix stability, however, are based on nearest-neighbor thermodynamic parameters. Rychlik described a computer program termed OLIGO to compute T_d based on nearest-neighbor thermodynamic parameters. The algorithm is set forth in Figure 1 of Rychlik.

Applicant mentioned OLIGO in the present Specification, page 5, lines 3-28. Applicant stated that PCR primer design software applications such as OLIGO work well for PCR primer design where relatively stringent conditions are employed. Unfortunately, noted Applicant, these conditions do not apply to oligonucleotide arrays, which are usually hybridized under relatively non-denaturing conditions, or to

antisense suppression of gene expression, which takes place *in vivo*. Applicant explained that oligonucleotide arrays can contain hundreds of thousands of different sequences and conditions are chosen to allow the oligonucleotide with the lowest melting temperature to hybridize efficiently. These "lowest common denominator" conditions are usually relatively non-denaturing and secondary structure constraints become significant. Accordingly, observed Applicant, the known approaches require new predictive methods that are capable of estimating the effects of oligonucleotide and target structure on hybridization. For these reasons, current algorithms for designing PCT primer oligonucleotides fail when applied to the problems of oligonucleotide array or antisense oligonucleotide design. Applicant observed further that, to date, the most effective approach for identifying oligonucleotides with good hybridization has been an empirical one.

Rychlik does not anticipate the claimed methods because, among others, Rychlik does not disclose Applicant's claimed steps of forming clusters and selecting, for a cluster, a hybridization oligonucleotide where the hybridization of a hybridization probe is predicted by the presence of a hybridization oligonucleotide in the cluster. The reference is completely devoid of any teaching in this regard and, as such, Rychlik does not disclose each and every element of the claimed invention.

The Office Action argues that Rychlik is noted as evaluating palindromic oligonucleotide segments. The Office Action contends that Rychlik describes self-complementary palindromes and refers to these as clusters of oligonucleotide sequences. The Office Action asserts that the instant claims are not limited as to the size, number, or complexity of the cluster identification. Thus, concludes the Office Action, Rychlik anticipates the claimed invention.

Applicant submits that the parameters discussed and utilized in Rychlik do not anticipate or suggest the cluster identification approach of the present invention. Such parameters are, for example, ones that may be used in step (b) of Claim 1. As recited in the claims, oligonucleotides are evaluated by at least one parameter and a subset of the evaluated oligonucleotides is selected. Then, oligonucleotides in the subset, not individual nucleotides within the oligonucleotides, are identified that are clustered along a region of the nucleotide sequence. Thus, in the evaluation according to certain parameter(s), some of the oligonucleotides of the original set of oligonucleotides are selected and others are rejected.

The above may be better understood with reference to pages 46-49 of the present Specification. On the basis of the parameters of T_m and ΔG_{MFOLD}, certain oligonucleotides at various positions along the nucleotide sequence complementary to target nucleotide sequence were rejected (shown with lines drawn through in the parameter columns). The remaining oligonucleotides are examined for clustering. On page 49 a cluster is shown having 13 contiguous oligonucleotides. As indicated on page 49, lines 4-5, any or all of the oligonucleotides in this cluster may be evaluated experimentally. Accordingly, in the above example, after the oligonucleotides are filtered according to predetermined parameter values, some oligonucleotides remain and others are rejected. The remaining oligonucleotides are viewed according to order of position along the nucleotide sequence and clusters of oligonucleotides are identified. In accordance with the invention, oligonucleotides that fall within the clusters are the ones of choice for further evaluation as hybridization probes. As mentioned earlier, Applicant has discovered that the clusters tend to contain oligonucleotides having a higher probability of showing good hybridization than oligonucleotides that are not in clusters.

As a result of the present invention, only a fraction of the potential oligonucleotide probe candidates are synthesized and tested. This is in sharp contrast to the known method of synthesizing and testing all or a major portion of potential oligonucleotide probes for a given target sequence (Specification, page 42, lines 28). Such an approach is particularly important in the area of array analysis of target nucleotide sequences. As explained in the Specification (page 5, lines 17-28), oligonucleotide arrays can contain hundreds of thousands of different sequences and conditions are chosen to allow the oligonucleotide with the lowest melting temperature to hybridize efficiently. These conditions are usually relatively non-denaturing and secondary structure constraints become significant. Arrays are generally utilized under relatively non-denaturing conditions in contrast to, for example, PCR conditions, which tend to be strongly denaturing.

Rychlik does not disclose or suggest the clustering approach employed in the present invention. Rychlik discusses several parameters for filtering oligonucleotides as part of a computer program called OLIGO. The program computes T_d values based on nearest-neighbor thermodynamic parameters and determines self-complementarity of the oligonucleotide, the presence of palindromes in the nucleic

acid sequence and the presence of alternative (non-target) sites for the oligonucleotide within the nucleic acid sequence. Rychlik (page 8454) indicates that the choice of oligonucleotide that anneals to all or part of a palindromic sequence for the nucleic acid in question would cause problems for double-stranded sequencing. Consequently, Rychlik uses palindromic evaluation of oligonucleotides to exclude them from consideration, not to include them for further analysis (page 8550, lines 8-10). The determination of self-complementarity involves repositioning in a selected oligonucleotide probe to determine base pairing. The length of base pairing of nucleotides within the oligonucleotide probe is determined. Oligonucleotides are included where the length of a continuous duplex is equal to or higher than a pre-set minimum. The discussion on page 8544, Fig. 1A, relates to the determination of self-complementarity, not to the palindrome determination as set forth at the bottom of page 8545 of the reference. According to the method of Rychlik, oligonucleotides are eliminated from consideration because of palindromes in the target sequence whereas oligonucleotides are included for further consideration in the self-complementarity approach. On page 8545 Rychlik notes that the program used is not intended to predict secondary structure for large nucleic acids (lines 22-23). In all of the parameter approaches of Rychlik, oligonucleotides are evaluated on an individual basis and, based on such analysis, individual oligonucleotides are chosen or discarded. This is evident, for example, in Figure 3 and the accompanying discussion on pate 8549.

There is no recognition in Rychlik that the hybridization of a hybridization probe is predicted by the presence of a hybridization oligonucleotide in a cluster. Such oligonucleotides are selected for further analysis. Rychlik does not disclose or suggest such an approach.

Claims 1-10, 15, 16, 18, 20, 21, 23, 28, 37, 39, 40, 98 and 100 were rejected under 35 U.S.C. § 102 (b) as being anticipated by Mitsuhashi, *et al.* (U.S. Patent No. 5,556,749) (Mitsuhashi). Mitsuhashi discloses a computerized method for the design of oligonucleotide probes based on the GenBank database of DNA and mRNA sequences and the examination of candidate probes for specificity of commonality with respect to a user-selected experimental preparation. Two models are available: a Mismatch Model that employs hashing and continuous seed filtration and a H-Site

Model that analyzes candidate probes for their binding specificity relative to some known set of mRNA or DNA sequences.

Mitsuhashi does not anticipate the claimed methods because, among others, Mitsuhashi does not disclose Applicant's claimed steps of forming clusters and selecting, for a cluster, a hybridization oligonucleotide where the hybridization of a hybridization probe is predicted by the presence of a hybridization oligonucleotide in the cluster. The reference is completely devoid of any teaching in this regard and, as such, Mitsuhashi does not disclose each and every element of the claimed invention.

Rejection under 35 U.S.C. §103

Claims 1-3, 5-10, 15, 17-22, 98-101 were rejected under paragraph (a) of the above code section as being unpatentable over Hyndman taken in view of Southern (U.S. Patent No. 5,700,637). The Office Action argues that it would have been obvious to someone of ordinary skill in the art at the time of the invention to practice Hyndman with Southern electronically transfer identified sequences (i.e., data) to an oligonucleotide array manufacturing system (i.e., computer) since Hyndman indicates the application of HYBsimulator to "design optimally specific DNA probes for dot blots, Southern blots, Northern blots, etc. "(i.e., oligonucleotide arrays).

Hyndman is deficient in not disclosing or suggesting forming clusters and selecting, for a cluster, a hybridization oligonucleotide where the hybridization of a hybridization probe is predicted by the presence of a hybridization oligonucleotide in the cluster. Southern does not cure these deficiencies because Southern does not disclose or suggest such a feature. Furthermore, the combined teachings of Hyndman and Southern do not suggest this feature of the claimed invention. Accordingly, the presently claimed invention would not have been obvious to one of ordinary skill in the art at the time the invention was made.

Claims 1-40 and 98-101 were rejected under paragraph (a) of the above code section as being unpatentable over Rychlik in view of Mitsuhashi or Hyndman further in view of Southern further in view of the Chemical Rubber Co. Handbook of Chemistry and Physics (the "Handbook") and Kress, *et al.* (Kress). Rychlik, Mitsuhashi, Hyndman and Southern are deficient as discussed above because none of these references teaches, either individually or in combination, forming clusters

and selecting, for a cluster, a hybridization oligonucleotide where the hybridization of a hybridization probe is predicted by the presence of a hybridization oligonucleotide in the cluster. Furthermore, neither the Handbook nor Kress cures this deficiency because neither reference teaches or suggests the aforementioned claimed subject matter and, thus, the combined teachings cannot teach or suggest such a feature.

Conclusion

Claims 1-25, 27-40 and 98-101 satisfy the requirements of 35 U.S.C. §§112, 102 and 103. A Terminal Disclaimer has been approved. Allowance of the above-identified patent application, it is respectfully submitted, is in order.

Respectfully submitted,

Theodore J. Leitereg
Attorney for Applicant
Reg. No. 28,319

Agilent Technologies, Inc.
Legal Department, M/S DL429
Intellectual Property Administration
P.O. Box 7599
Loveland, CO 80537-0599
(650) 485-2386